Review Article

Combretastatin A-4 and its Analogs in Cancer Therapy

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ABSTRACT

Combretastatin A-4 (CA-4) is an anti-mitotic agent that is gaining rapid recognition among cancer biologists and clinicians as one of the newer vascular disrupting agents, (VDAs) for cancer therapy. CA-4 belongs to a group of tubulin binding natural products called combretastatin, derived from the African Bush Willow, *Combretum caffrum*. Due to CA-4's *in vivo* efficacy, a number of analogs of CA-4 have been synthesized to maximize its solubility and bioavailability. Combretastatin A-4 phosphate is a more soluble form of CA-4 that has successfully completed phase I trial and is currently under phase II/III trials for treatment of acute myelogenous leukemia and relapsed ovarian cancer. This review attempts to highlight the various CA-4 analogs that have been synthesized and their effectiveness in clinical trials as VDAs.

Keywords: Combretastatin A-4, Microtubule, Vascular Disrupting Agents, Angiogenesis.

INTRODUCTION

Recent understanding of tumor tissue architecture and environment, along with cellular processes of metastasis and angiogenesis, has lead to development of targeted cancer therapies that kill tumor cells indirectly by attacking tumor vasculature. [1, 2] There are two prominent strategies of targeting tumor vasculature, anti-angiogenesis & anti-vasculature, each of which has been explored to some degree of success. [3] Whereas, anti-angiogenic approaches are designed to prevent the neovascularization of tumors so that no new blood vessels may form, anti-vascular approaches target the established tumor vasculature causing hemorrhagic necrosis and cell death.[4, 5] Compounds targeting tumor vasculature are known as Vascular Disrupting Agents (VDAs). These compounds prove more advantageous compared to cytotoxic compounds. Cytotoxic compounds must reach every tumor cell in order to kill them whereas an indirect damage to tumor vessels results in the death of many tumor cells simultaneously by depriving them of nutrients and oxygen.[6] The indirect targeting also avoids the high risk of acquired drug resistance as is seen with cytotoxic compounds. [7]

Tubulin polymerization inhibitors are a class of small molecule VDAs that induce hemorrhagic necrosis of solid tumors.[8] Although two most common and historically oldest tubulin inhibitors: vinca alkaloids and colchicine are known for their tubulin binding and anti-cell proliferative effects, their anti-vascular effects were often only observed at doses approaching or exceeding maximum tolerated doses (MTD). Combretastatin A-4 (CA-4) and its more soluble analog combretastatin A-4 phosphate (CA-4P), are tubulin polymerization inhibitors that are able to disrupt tumor vasculature at doses that are well below MTD. As an antimitotic, CA-4 is able to induce apoptosis by cell-cycle arrest . [9,10] The antimitotic mechanism of action of CA-4 is well documented and is due to its ability to disrupt microtubule dynamics and hence mitotic spindle by binding to colchicine binding site in a tubulin dimer.[11, 12] As a VDA, CA-4 causes occlusion of tumor vasculature resulting in hypoxia-driven necrosis focused on the core of the tumor. [13]

Chemical structure of CA-4 is shown in Figure 1 along with structure of other microtubule binding agents. A number of variants of this structure have been synthesized and tested. It has been generally accepted now that a common structural motif for the analogs to remain active consists of two phenyl systems: a 3,4,5-trimethoxyphenyl and a 4-methoxy-3-X-substituted phenyl system separated by a alkene bridge that retains a *cis*-configuration. In this article we focus on the analogs of CA-4 that have been synthesized and are currently under clinical investigation.

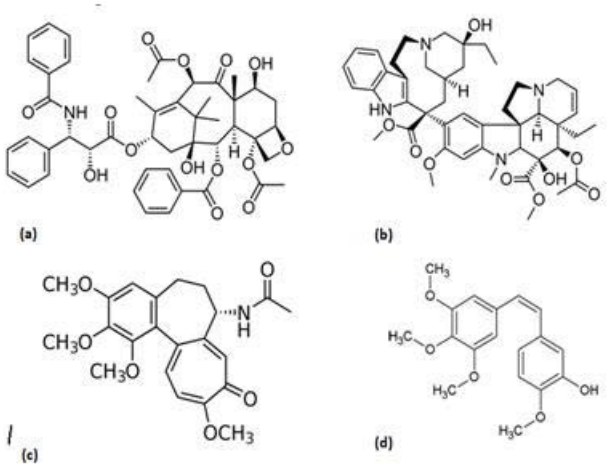


Figure 1: Chemical structure of microtubule binding agents: (a) Taxol (b) Vinblastine (c) Colchicine (d) Combretastatin A-4

Combretastatin: Natural Product with Anti-mitotic and Anti-vascular effects

Newman and Cragg, among others, have established that well over half of the anticancer drugs introduced in the last three decades have originated from a natural product or can be traced back to a natural source.[14] Combretastatins are a class of stilbenoid phenols that were isolated over two decades by Pettit et al. from a bush willow tree *Combretum caffrum.*[15] *Combretum caffrum* belongs to Combretaceae family of shrubs found in South Africa, principally in the Eastern Cape and Transkei to Natal. [16] The Xhosa people of South Africa have been reportedly using *C. caffrum* for decades and longer for the treatment of many ailments ranging from heart and worm remedies, wound dressings and scorpion sting. [18] The natural products isolated by Pettit et al., from *C. caffrum,* exhibited strong biological activity as inhibitors of tubulin polymerization with potency both *in vitro* on cancer cell lines[18] and *in vivo* as vascular targeting agents. [19] Subsequent studies found that these isolates consist of a number of series with different medicinal chemistries. Combretastatin A-4 (CA-4) and combretastatin A-1 (CA-1) have been the most prominent leads for therapeutic applications of the combretastatin A series.[20, 21] There exists a B, C and D-series of the combretastatins that have been isolated as well. [22-24]

The antimitotic effects of combretastatins are due to inhibition of the function of microtubules. Microtubules are well validated target in for cancer therapy. Microtubules are formed by α-tubulin and β-tubulin heterodimers. The polymerization and depolymerization of tubulin has been shown to regulate microtubular dynamics. [25] This plays a vital role in the formation of the mitotic spindle and in cytokinesis at the end of mitosis.[26] Molecules binding to tubulins disrupt microtubule dynamics, inducing cell cycle arrest and apoptosis. There are three binding sites within tubulin heterodimer known as taxol [27], vinca alkaloid [28] and the colchicine[29] binding sites. CA-4 binds to tubulin using the colchicine site. Binding to tubulin heterodimer results in disruption of the heterodimer and thus inhibition of their polymerization into microtubules. [30] This further causes formation of irregular mitotic spindles and metaphase arrest of mitotic cells. Colchicine is known to be toxic and has few therapeutic uses but the binding of CA-4 to the colchicine site of tubulin has shown potent cytotoxicity against cancer cells including MDR cell lines. [31,32]

Not only is CA-4 considered an antimitotic agent, but it also exhibits an anti-vascular and anti-angiogenic effects and is commonly referred to as a VDA. Solid tumors are usually able to proliferate using existing vasculature as well as through angiogenesis but changes in the morphology of the endothelial cells lining microvessels begin to destroy the tumor seemingly from within. [33] CA-4 is shown to cause direct damage to the vasculature which in turn prevents angiogenesis by inducing morphological changes to endothelial cells. CA-4 disrupts the endothelial cell–specific junctional molecule vascular endothelial-cadherin (VE-cadherin) and so the activity of the VE-cadherin/β-catenin/Akt signaling pathway, which may result in the inhibition of endothelial cell migration and capillary tube formation. These anti-vascular effects target the core of the tumor. This is unlike many other anti-vascular treatments in that other treatments target only the peripheral cells leaving the core of the tumor unaffected and promoting multi drug resistance (MDR) as a result. [34, 35] CA-4 is therefore more effective than other treatments in MDR patients. CA-4 displays highly specificity within the tumor when distinguishing between normal and tumor endothelial cells although the mechanism behind this is unknown. These effects are also seen at doses much lower than the maximum tolerated dose (MTD) leading to lower risk of toxicity.

Combretastatin: A-4 Analogs

CA-4 has been a very attractive lead molecule for the treatment of tumors and therefore many synthetic analogues of CA-4 have been created in order to improve upon CA-4's cytoxicity and inhibition of tubulin polymerization.[36-38] These structure-activity relationship (SAR) studies have revealed that the *cis* configuration of the ethene bridge and the 3,4,5-trimethoxyphenyl group on ring A are fundamental to maintaining a functional compound.[39] The 4-methoxy group appears important for cytotoxicity and therefore does not take easily to structural modifications whereas the 3-hydroxy group is more tolerant of these structural modification[40]. While scientific literature is full of CA-4 synthetic analogs, we have focused our discussion here on some of the most promising analogs that show significant colchicine binding, anti-microtubule and anti-cell proliferative activity. In these studies (a) colchicine binding activity refers to ability of the analog to compete with radioactive colchicine for binding to colchicine site on purified tubulins. (b) microtubule activity refers to an *in vitro* assay which measures degree of polymerization of microtubules from purified tubulins using turbidometric based assay in presence and absence of the analog (c) cell viability activity refers to ability of the analog to inhibit the proliferation of cancer cells in cell culture models. For the purposes of our discussion, if more than one IC_{50} values were available for different cancer cell lines, we calculated the mean of these values to give a representative trend. Table 1 summarizes these findings and Figure 2 represents a common structural motif of CA-4 analogs discussed here.

One of the most significant studies is by Pettit et al.[41] who synthesized a series of *cis*- and *trans*-stilbenes related to CA-4 with a variety of substituents at the 3'-position of the B-ring. The *trans*-stilbenes had little to no activity against the several cancer cell lines in which they were evaluated. However, in terms of inhibition of cell growth and tubulin polymerization, the dimethylamino (#1, Table 1) and bromo (#2, Table 1) *cis*-stilbenes were most potent with biological activity similar to that of CA-4. Separately, Cushman et al.[42]

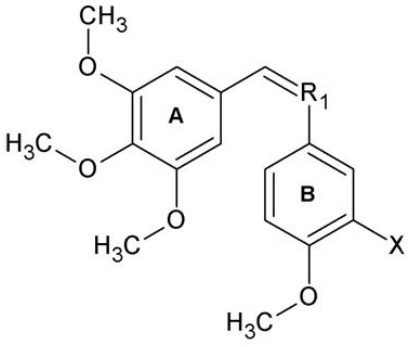
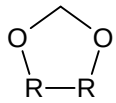
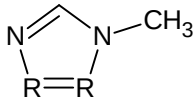


Figure 2: Common Structural Motif of CA-4 Analogs

Table 1: Favorable B-ring substitutions and Bridge modifications of CA-4

(* Refer to Figure 2 for structural motif that displays chemical positions of R_1 and X)

Compound	R_1*	X*	Colchicine Displacement Activity (IC_{50}-μM)	Tubulin Binding Activity (IC_{50}-μM)	Cell Viability Activity (IC_{50} – μM)	Reference
CA-4	-OH	-CH	0.14	2.5	0.007	20
1	-NMe_2	-CH	-	-	0.09	41
2	-Br	-CH	-	1.1	0.002	41
3	-OMe	-CH	5	8.8	0.06	42
4	-Cl-4-OMe	-CH	5	3.5	0.017	42
5	-Cl	-CH	5	4.8	0.01	42
6	-Br	-CH	5	3.1	0.006	42
7	-N_3	-CH	33	-	5.2	43
8	-F	-CH	-	-	0.004	44
9	- $OB(OH)_2$	-CH	79	1.5	-	45
10	-NH_2	-C	-	3	195	46
11	-OH	-NH^+Cl^-	-	-	11	47
12	-OH		-	-	4	48
13	-OH		-	-	31	49

prepared a series of CA-4 analogs, both *trans* and *cis*-stilbenes. The *trans*-stilbenes again had no activity while the *cis*-stilbenes ((#3-6, Table 1) were all potent inhibitors of tubulin polymerization with cytotoxicity comparable to that of CA-4. Pinney et al. [43] synthesized aryl azide analogs of CA-4. While substitutions of the 4'-carbon did not yield good activity, the replacement of the 3'-hydroxyl group of CA-4 with an azido moiety (#7, Table 1) demonstrated excellent *in vitro* cytotoxicity against human cancer cell lines. The most active fluoro analog of CA-4, synthesized by Lawrence et al., was found to be 3-deoxy-3-fluoro-combretastatin A-4 (#8, Table 1) which retained cytotoxicity.[13] A boronic acid (#9, Table 1) moiety was synthesized by King et al.[44] and it was suggested via docking studies that the *cis*-isomer would be a potent inhibitor of the colchicine binding site. Last but not least, Ohsumi et al. [25] synthesized a number of compounds to test their effect against murine solid tumors. The most promising *in vitro* was also potent (#10, Table 1) *in vivo*, although in a hydrochloride form.

In order to see the effect of bridge modification on activity of CA-4, Cushman et al. [47] synthesized a series of water soluble aniline salts and were tested against human cancer cell lines. The most promising compound (#11, Table 1) showed inhibition of tubulin polymerization and cytotoxicity. Shirai et al. [48] synthesized compound 12 (Table 1) with as a chirally preorganized derivative of CA-4. Tubulin polymerization inhibitory activity was strong and measured at 20 times more potent than vincristine. Wang et al.[49] synthesized a number of heterocyclic bridge replacements with relatively a small methyl group resulting in a 6-15 fold loss in cytotoxicity. The oxazole-containing compounds did show antitubulin activity

comparable to CA-4, with compound 13 (Table 1) showing the strongest potency.

In addition to the analogs shown in Table 1, P. Liou et al.[50] synthesized a series of 3-aminobenzophenone compounds that showed potent cytotoxic and tubulin polymerization inhibition effects. Their study further revealed that the methoxy groups should preferably be located at the C-4 position rather than C-5 or C-6. Further examples of CA-4 bioisosteres include ethers[51], sulfonamides[52-54], sulfonates[55], amine or amide derivatives [56, 57], cyclopentanes[58], pyrazoles[59], thiazoles, triazoles[60], tetrazoles[61], imidazoles, furans, furanones, furazans[62], dioxolanes, thiophenes[63], thiadiazoles[64] and indoles. [65, 66] These moieties attempt to maintain the spatial arrangement of the aromatic systems while successfully maintain potent cytotoxicity and tubulin binding.

Whether the analogs were successful or not, a number of trends and observations were made that should help guide new SAR studies. *Cis*-stilbenes are generally the best inhibitors for tubulin polymerization[67], and it has been indicated that their activity is not due to a totally planer conformation. [68] An increase in steric bulk at the 4-position of the B-ring can result in a decrease in activity. [69] The *cis* oriented double bonded bridge can be replaced by ring systems such as dioxolane, tetrazole, thiazole, indole, thiophene, imidazole, pyrazole, isoxazole, oxazole, cyclopentone, azetidionone, pyridine, furazan or oxadialine. These rigid analogs still maintain activity and some have shown oral availability. In conclusion, the common structure characteristics for combretastatin to be active follow a 3,4,5-trimethoxyphenyl and a 4-methoxy-3-X-substituted phenyl system which is separated by a two atom bridge that retains *cis*-configuration.

Clinical Studies

Due to their good solubility, safety profile and efficacy three of the CA-4 analogs are currently in clinical trials: CA-4P, Oxi4503 and AVE8062 (Figure 3).

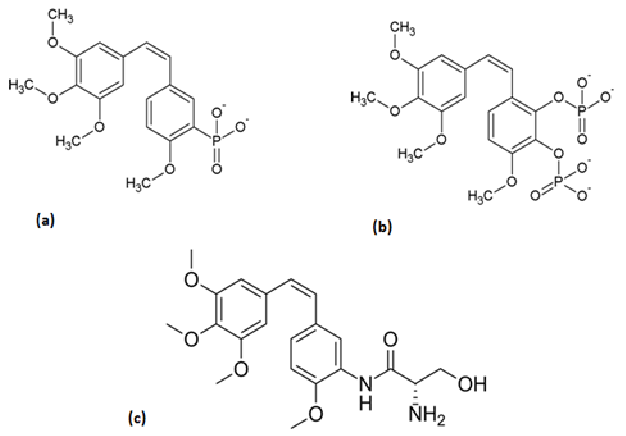


Figure 3: Chemical structure of CA-4 analogs in clinical trials: (a) CA4-P (b) Oxi-4503 (c) AVE-8062

CA-4, although a potent anti-vascular agent, binds to plasma proteins in the blood thereby reducing its cellular permeability reducing the amount of drug found at the target site and decreasing its effect. Due to the problems encountered in the pharmacokinetics of CA-4, a disodium phosphate salt of CA-4 called CA-4P was developed. CA-4P has been shown to have increased organic phase solubility meaning that it will reach the target more readily than CA-4. The increased polarity of CA-4P however, may be envisioned as a drawback in that it will have more difficulty crossing membranes. However, since tumor vasculature in general consists of leaky capillaries, CA-4P does not seem to have any major difficulties in passing through tumor vasculature. Once, inside the cells, CA-4P is dephosphorylated to CA-4 and effects tumors in the same way.[10] CA-4P is currently in phase II/III trials as a combination therapy with paclitaxel and carboplatin. Phase I trials determined the Maximum Tolerable Dose (MTD), safety and pharmacokinetic profile of CA-4P on a single dose i.v. schedule. In dose escalation studies, tumor pain was observed with Dose Limiting Toxicity (DLT) around 60mg/m2 with reported cardiopulmonary toxicity (dyspnea, hypoxia and syncope) at 75 mg/m2. Use of Dynamic Contrast Enhanced-Magnetic Resonance Imaging (DCE-MRI) showed decline in gradient peak tumor blood flow. [70] CA-4P was found to be well-tolerated by kidney or muscle and vascular disrupting activity was confirmed using Position Emission Tomography (PET) measurements. Some patients showed disease stability for 2 cycles while dose-dependent reductions in tumor perfusion and blood volume were rapidly produced.[71] Another trial, a single-dose study, concluded that patients with known coronary artery disease, multiple or otherwise should not have eligibility for future trials due to prolonging of the QTc interval. [72] Combination trials of CA-4P with carboplatin and paclitaxel (or both), showed significant response, especially with relapsed platinum resistant ovarian cancer. Patients were treated with CA-4P (63mg/m2) 18-20 hours prior to treatment with paclitaxel (175mg/m2) and carboplatin. 32% of all patients showed some response with adverse effects demonstrating neutropenia and hypertension along with those expected with paclitaxel and carboplatin (fatigue, nausea, alopecia, diarrhea, and reversible ataxia). [25] Other ongoing trials are currently exploring anaplastic thyroid cancer as well as chemotherapy naïve lung cancer (described at http://clinicaltrials.gov).

Oxi4503 is derivative of CA-4P that contains an additional phosphate group to further enhance its solubility (Figure 4); marketed by OXiGENE Inc. Phase I evaluation of Oxi4503 established the safety, tolerability and pharmacokinetics of the drug, along with determining its MTD[74]. Common adverse effects included tumor pain, nausea, hypertension, fatigue and myelosuppression. Dosage related dose-limiting toxicities (DLTs) were seen at 15.4 mg/m2. In 2008, the trials found a dose-dependent linear increase in peak plasma concentrations of Oxi4503. [75] Further results from a phase II study in

patients with advanced ovarian cancer showed partial response at two dosages: 11 and 14 mg/m2. These dosages may only be tolerated when used with a prophylaxis to prevent hypertension. 67% of evaluated patients also showed DCE-MRI changes consistent with strong VDA activity. [76] A new trial using Oxi4503 in combination therapy is currently recruiting for patients with relapsed and refractory acute myelogenous leukemia (AML) and myelodysplastic syndromes (MDS) while an on-going phase Ib/II of Oxi4503 as a monotherapy is expected to conclude in July 2011 (described at http://clinicaltrials.gov)

AVE8062 or ombrabulin, marketed by Sanofi Aventis is an analog of CA-4 with -(NH-CO-NH_2OH) modification in the B-ring(Figure 5). AVE8062 has completed a phase I trial combined with docetaxel in which AVE8062 was administered 24 hours prior to docetaxel. The most common tumor adverse effects included neutropenia and thrombosis. The MTDs for the 48 patients (treated in 2 cohorts) were 35/75 mg/m2 of AVE8062/docetaxel respectively for the first cohort and 30/100 mg/m2 for the second. [77] There are currently 4 active phase I trials focused on advanced solid tumors in combination with cisplatin or bevacizumab and another using radio labeled AVE8062 (described at http://clinicaltrials.gov).

CONCLUSION

CA-4 has played a leading role in the evolution of the field of vascular targeting. Several of its analogs are swiftly moving through clinical trials with strong results. Other analogs may begin the early stages of preclinical development in the near future. Generally low MTDs and non-overlapping toxicities with traditional chemotherapy and radiotherapy have been observed in the CA-4 based VDAs developed thus far. More recently developed analog VDAs such as AVE8062 and Oxi4503 are more potent at lower doses than the MTDs. These analogs have shown promise in the treatment of tumors, especially in combination therapies with other anticancer agents. Tumor regression has been observed consistently and there is a trend for increased effectiveness as tumor size is increased. This is most likely to be due disruption of the newly formed vasculature by these agents. It will be important to determine the anti-vascular mechanism of action of CA-4 in detail in order to better understand some of the adverse effects and toxicities that are observed in patients. There is also a need to further advance structure-activity studies of CA-4 based analogs in order to generate orally bioavailable compounds.

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